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THE BRITISH VETERINARY JOURNAL

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EDITORIAL

ANIMALS AND CHILDREN

Men and the domesticated mammals are built according to the same basic design. In spite of this there are great differences in form and function for, during the process of time, modifications have been made in order to fit the various animals to their environment, sentient reactions, and mode of life. Speaking in broad biological terms probably the greatest matter of difference between these animals and man is centred in the nervous system for homo sapiens is blest, there are some who say he is cursed, with the qualities of thought and reason and speech. All the animals are sentient to a great degree. They can experience fear, flush with anger and be imbued with greed, jealousy and covetousness, and these things may so upset the even balance of their lives that their emotional reactions are manifested in acts of physical strife and even cruelty. Under other circumstances emotional response can be expressed in the tender attributes of solicitude and kindness, or it may lead to the exhibition of the qualities of courage and endurance. Since the very dawn of civilisation, which was conceived when man acquired fortuitously the power to think and reason and the ability to express his thoughts in coherent speech, it has been recognised, but not always implemented, that the path to a higher state depended upon the control and elimination of evil things and the enhancement of those which were of good intent.

Some men are born to be kind and gentle, others can be trained or directed on to the right path and there are some who will fail to respond to any approach. There is evidence to show that the average man is more gentle in nature and kinder in spirit than his ancestors were. Acts of cruelty which give cause for the expression of horror and consternation in 1959 would have occasioned little or no comment a hundred years ago. On the surface it would seem that this mellowing of the spirit has been actuated by the advancement of the standard of general education and the spread of civilisation. Such a premise is not correct by any means. Acts of cruelty are not perpetrated always by those from the ranks of the vulgar untutored mob. The training of the mind and

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the accumulation of knowledge does not necessarily imply that the culture and enhancement of the things of the heart or spirit advance in due proportion. The reverse may occur. An excited and enthusiastic searcher for factual information can become so obsessed with his task that he may ignore or sweep aside all those things which halt or impede the progress to his ultimate goal. These can include any thought for the welfare of his fellows and especially those things which stem from spirit or sentiment.

The ideal state would be one in which the advancement of knowledge in the philosophical and scientific spheres of endeavour should run parallel with the culture of the sentient nature from which stems the gracious qualities of kindness and compassion. If such a state of affairs could be achieved most of our troubles would vanish. A child is not born with a store of knowledge. He has to acquire it as he passes through his life. The seed is sown at his mother's knee and in his school or college. The training he receives fits him to run under his own steam thereafter if the urge of desire be present. The culture of the spirit follows a similar course and proper training leads to good maturation.

One of the best ways to sow the seed of kindness is to encourage children to keep pet animals. Even in this mechanised age most children like to possess an animate pet on which they can lavish their affection. There is a great affinity between children and animals, they have much in common, they understand one another and their association is to mutual benefit. Love and kindness flow freely between them and these qualities being grafted into the child help him in turn in his contacts with his human friends.

Children are always anxious to know more about their pets and in those schools in which the elementary principles of structure and function are taught the biology sessions are enjoyed and serve a most useful purpose. There have been reports that, in some schools and under the influence of enthusiastic teachers, the children's inquiries into the structure and function of their pet animal has been extended so that they witness experiments on living animals and are encouraged to pursue their researches at home. This is a thing to be deplored. Enthusiastic teachers of this nature should satisfy their search for knowledge in other spheres, i.e. in research institutions where they would work amongst mature colleagues and under strict control. They should remember that children are simply animals in the process of being trained to fit them for adult life. Children have not a great fund of knowledge on which to draw and they accept a teacher's dictates as gospel. If the teacher can see no wrong in children performing experiments on living animals then the natural consequence is that the boys and girls will likewise accept this diction in all innocence and it is probable that their desire to keep a pet will not be excited by the joys of love and companionship but, in order to test the reactions of the poor dumb beast, by pseudo scientific methods.

THE MECHANISM OF IMMUNITY IN CANINE VIRUS HEPATITIS

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Introduction

The literature on canine virus hepatitis is quite considerable, but little is known of the mechanism of immunity in this infection.

During the past nine years, work has been done in this laboratory on various aspects of the mechanism of immunity and artificial immunisation in canine virus hepatitis. Some of the relevant experimental work and observations thereon are recorded in this paper.

Materials and Methods

Most of the experimental materials and techniques have been described in detail in preceeding papers (Larin, 1951 a and b; Larin and Orbell, 1956; Larin, Gaddum and Orbell, 1957; Larin, 1958 a). Wherever other materials or techniques have been used, these will be described under appropriate headings below.

Host-Virus Relationships

Fig. 1 schematically shows data derived from a litter of four month old puppies which have acquired canine virus hepatitis spontaneously. This litter of puppies came

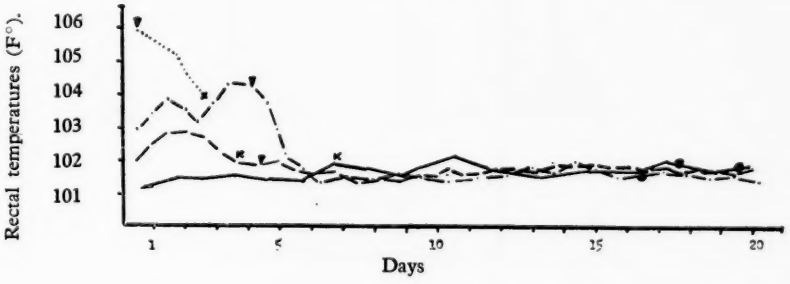

FIG. 1

Different forms of naturally acquired canine virus hepatitis in four weaned puppies belonging to one litter.

Puppies: No. 7 .....; No. 12 —.—; No. 10 ——; No. 8 ———;
Blood in faeces ??; Kerato-conjunctivitis K; Antibody ●; Death X.

under observation on the day it was noticed that puppy No. 7 had passed blood stained stools (see Fig. 1). In other respects this puppy was alert and active in spite of a temperature of 105.8°F. On the third day the puppy showed signs of circulatory collapse and died. Post mortem and histopathological examination of the puppy showed

the pathological features of acute virus hepatitis, and the virus was transmitted to susceptible puppies.

As is shown in Fig. 1 one of the other puppies of this litter (No. 12) developed the disease in a severe non-fatal form; one, (No. 10) in a mild form; and the other three puppies (Nos. 8, 9, 11), which are represented in Fig. 1 by a typical example i.e. puppy No. 8 acquired the inapparent form. The antibody in these puppies after convalescence, was detected by the complement fixation reaction.

Having illustrated in Fig. 1 an example of what can be considered four typical forms of canine virus hepatitis, it is intended to show here the incidence of these and also the distribution of tissue lesions according to the severity of illness. It is for this purpose that Fig. 2 has been made to suggest that canine virus hepatitis may be regarded as a typical representative of those endemic virus infections, which, in the majority of cases take either a mild course or, alternatively may be clinically inapparent (symptomless).

The use of the terms " mild " or " inapparent " infection may erroneously suggest that the organs are not affected, Fig. 2 shows, however, that specific liver lesions have been detected histologically in the majority of dogs with the inapparent infection.

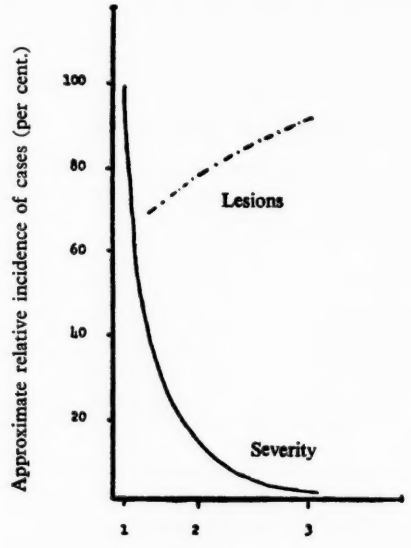

FIG. 2

Distribution of cases of canine virus hepatitis (natural infection) according to the clinical severity. Subclinical infections—1; Severe non-fatal form—2; Fatal form—3.

Activity of the Virus in the Dog after Convalescence

One of the interesting facts that emerged during these investigations was that residual lesions in the liver from clinically recovered dogs were very pronounced, showing a considerable delay in tissue restitution. Since the tissue contained also infective virus the question naturally arose whether the lesions were in fact " residual ".

We have found on a few occasions that dogs affected with virus hepatitis had electrophoretic patterns of the serum protein showing abnormal increases in alpha and beta globulin, and, after this chance observation, it was decided to investigate whether the electrophoretic patterns of serum proteins are of some significance in determining the virus activity in the body. For this purpose we have examined the electrophoretic patterns of the peripheral venous blood sera from 25 puppies, 2-4 months old, which

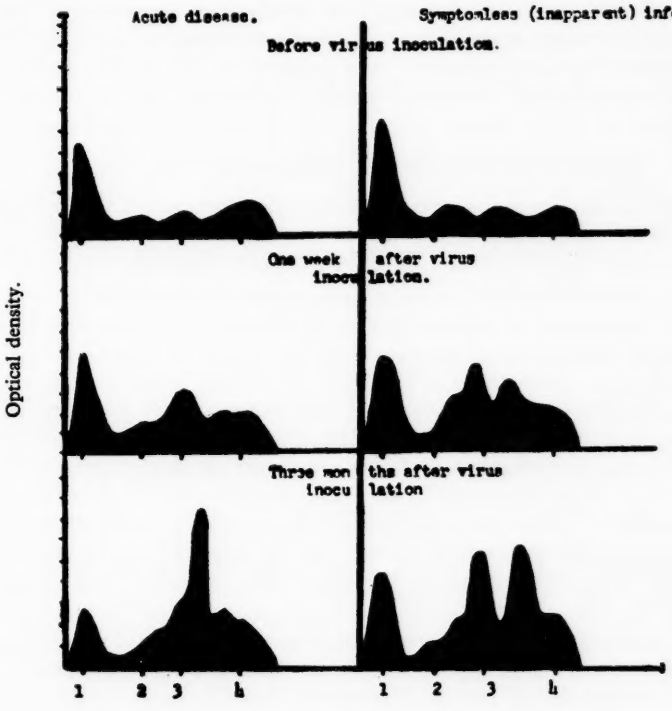

FIG. 3

Typical serum patterns obtained with paper electrophoresis.
1—Albumin; 2, 3, 4—Alpha, Beta, Gamma globulin.

had been inoculated with canine hepatitis virus. Abnormal electrophoretic patterns of the serum proteins were found in all puppies which developed acute virus hepatitis. The puppies showed marked rise in alpha and beta globulin or in both. In some, albumin was lowered as early as in the beginning of the disease, but, in the majority, albumin, while being at the normal level during fever, was considerably lowered either in the convalescence period or after complete recovery; gamma globulin in the majority of cases was elevated at a later stage of the infection. Similar changes in the protein pattern were also found in puppies who after virus inoculations developed symptomless (inapparent) infection.

The above electrophoretic data are represented in Fig. 3 by two cases of experimental infection (one acute and one inapparent).

From Fig. 3 it may be concluded that changes in the protein patterns represent some reflection of the active infective process. Although it would seem unwise to advocate that protein patterns which are shown in Fig. 3 are specific for canine virus hepatitis, the patterns suggest that the virus probably is considerably active in tissues of dogs with symptomless (inapparent) infections and also in dogs after convalescence. In the light of these findings, " residual " liver lesions which are commonly found in clinically recovered dogs, are possibly foci of virus proliferation in the altered milieu, where the immune mechanisms appear to be incapable of eliminating the virus. It must be emphasised however, that this conclusion is supported only by circumstantial evidence.

Differences in the " Normal " Resistance to Infection

The problem of immune barriers in canine virus hepatitis are closely linked to the problems of pathogenesis of the disease and especially to the sites of virus multiplication and the pathways of virus dissemination in the body.

Since it has been impossible to obtain certain types of information on both these aspects from spontaneous infections, experimental inoculation of dogs with the virus provided useful comparisons of the basic infection process. After injection of the virus—the observable phenomenon in dogs such as viraemia, serum antibody response and also pathological changes in fatal cases were remarkably similar to the events occurring in spontaneous infections. Probably the disease is pictured better as a sequence of virus multiplications beginning somewhere at sites of virus infection, spreading to the visceral organs, and in some cases to the central nervous system. The ability of the virus to spread in visceral organs is beyond any dispute, and the evidence that virus can multiply in the central nervous system comes from the pioneer works of Green (1934) and Rubarth (1947), the data of these authors being confirmed by many others.

Unfortunately it is very difficult to establish the exact pathway of virus dissemination from the peripheral sites of multiplication to the visceral organs. However, since the evidence is clear enough that the virus has the ability to enter the blood stream, this supports the idea that it probably invades the visceral organs by way of blood vessels.

Table 1 shows comparisons of the median incubation period after virus inoculations by various routes, as indicated by the occurrence of fever and by a rise of titres of the complement fixing antibody after convalescence.

Summing up the above experiment it can be concluded that the median incubation period in dogs, in which the disease developed in a clinical form, fever being taken as the indicator, was as short as 30 to 32 hours after intravenous, intramuscular and intraperitoneal inoculations, but it was 78 hours after subcutaneous inoculations, and 92 hours after intranasal virus instillation. Virus would therefore seem to reach the visceral organs after intramuscular or intraperitoneal inoculations as rapidly as after intravenous inoculations. The incubation period was considerably longer after subcutaneous inoculations or intranasal virus instillations.

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For experiments which are illustrated in Table 1 puppies, 3-5 months old which showed, before virus inoculations, no presence of the complement fixing antibody to canine hepatitis virus in the peripheral blood were used. It appears from the foregoing table, that despite the fact that all the 65 experimental puppies were serologically negative, as was shown by the complement fixing reaction, the degrees of resistance was different in individual puppies. Whilst some of them developed the clinically recognisable disease after virus inoculations, the reaction of the others was clinically inapparent and could be detected only serologically by the appearance of the complement fixing antibody in the peripheral blood.

TABLE 1

The Median Incubation Period in Acute Cases of Rubarth's Disease which has been Produced Experimentally in Young Dogs.

Inoculations		Clinically recognisable disease (As indicated by fever)		Number of dogs which developed inapparent infections (As indicated by the rise of the complement fixing antibody)
Route	Number of dogs	Number of dogs	Incubation period (hours)	
Intra-venous	12	5	32	7
Intra-muscular	6	2	30	4
Intra-peritoneal	23	6	30	17
Subcutaneous	18	3	78	15
Intranasal instillation	6	1	92	5
Total	65	17	—	48

The Possible Role of the Complement Fixing Antibody in Immunity

It is known (a) that varying severity of the disease makes little difference to the subsequent immunity, and (b) that dogs after convalescence are equally immune to the virus on challenge inoculations independent of whether these are made before the appearance of the complement fixing antibody or within or after the period of its persistence in the peripheral blood (Larin, 1953).

In an attempt to study the role of the complement fixing antibody in immunity to the virus, the particulate complement fixing antigen was purified with the use of lanthanum acetate (Larin and Orbell, 1956) and the purified preparation was inoculated subcutaneously into 37 dogs as follows:

1. 10 puppies—3 months old which showed no presence of the complement fixing antibody in the peripheral blood.
2. 7 adult dogs which were serologically negative as above.
3. 20 adult dogs showing low titres of the complement fixing antibody in the peripheral blood.

The results of inoculation of the above dogs with the particulate antigen are shown in Fig. 4.

As can be seen from Fig. 4 the particulate antigen on inoculation to the puppies of group 1 produced the disease in a severe form with prompt recovery and with

uneventful convalescence. The complement fixing antibody was detected in the peripheral blood of the puppies on the 16th day after convalescence, and the puppies appeared to be immune to the virus on challenge inoculations which were made on the 21st day after convalescence. Thus it can be concluded (a) that the particulate antigen was infective, (b) that inoculations of the antigen stimulated the appearance of the complement fixing antibody in the peripheral blood, and (c) that the antigen inoculations rendered the puppies immune to subsequent challenge inoculations with the virus.

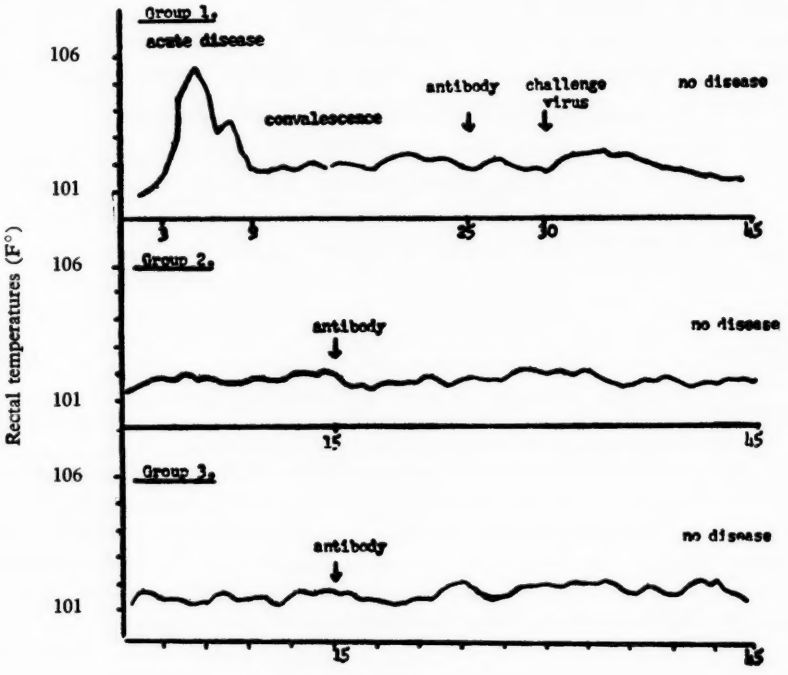

FIG. 4
Inoculation of dogs with the particulate antigen.

The adult dogs in group 2 which were also serologically negative, had not reacted clinically to inoculations with the particulate antigen. However, high titre of the complement fixing antibody appeared in the peripheral blood of these dogs 15 days after inoculations of the particulate antigen.

The third group of 20 dogs, which had before inoculation with the particulate antigen, low titres of the complement fixing antibody in the peripheral blood, gave the sharp antibody rise (see Fig. 4) which was detectable on the 15th day after inoculation with the antigen.

An attractive but unproved interpretation of these findings is that the particle of complement fixing antigen is identical with the virus particle and that the antigen possibly is a form of multiplying virus. Secondly, since the virus is able to survive in an immune host for considerable periods of time (Poppensiek and Baker, 1951; Larin and Orbell, 1956) the logical conclusion, if identity of the complement fixing and the neutralising antibody is assumed, would be that low levels of antibody which are undetectable by the complement fixation reaction made immediately after convalescence, are sufficient to arrest for some time proliferation of the virus, but not to bar it from the body. If this is the case, then it would appear that the usual sharp antibody rise two to three weeks after convalescence may represent the body's reaction to the emergence of active virus.

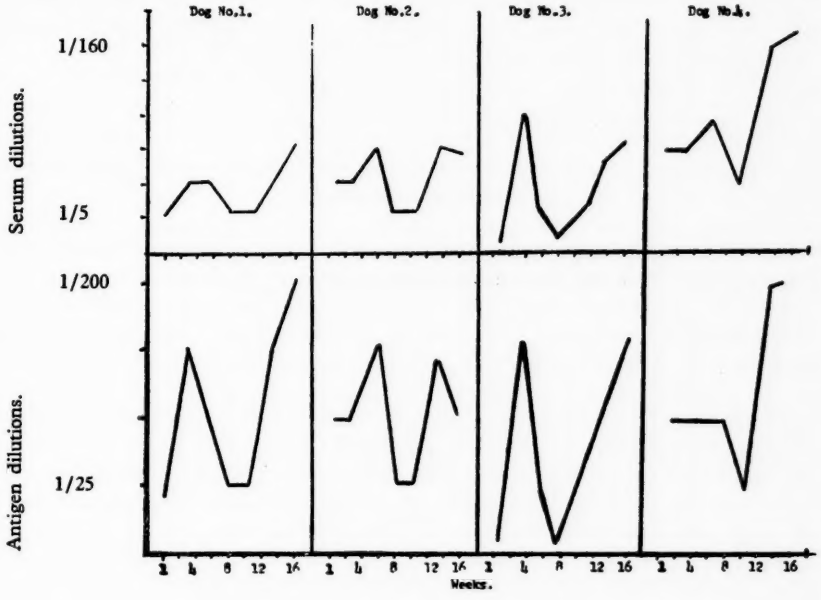

FIG. 5

Titrations of serum from dogs after convalescence.

The Naturally Operating Booster Effect

In anticipating the fact that canine hepatitis virus is able to persist in immune dogs, sera from dogs which have recovered from a mild form of the disease were examined at regular intervals by the complement fixation reaction. Fig. 5 shows in graphic form typical protocols of these experiments. Blood samples from the four dogs shown in Fig. 5 were withdrawn on nine occasions at intervals of two weeks and the specific complement fixation reactiveness of each serum was determined by (a) testing it in varying dilutions against the constant dilutions of virus antigen of 1:25, and also (b) testing each serum in the constant dilution of 1:5 against varying dilutions

of the virus antigen. The apparent agreement of both titrations which can be seen in Fig. 5 would seem to justify the conclusion that pronounced fluctuations in the specific complement fixing reactions of the sera must have been associated with the survival and multiplication of the virus in the body of immune dogs. The emergence

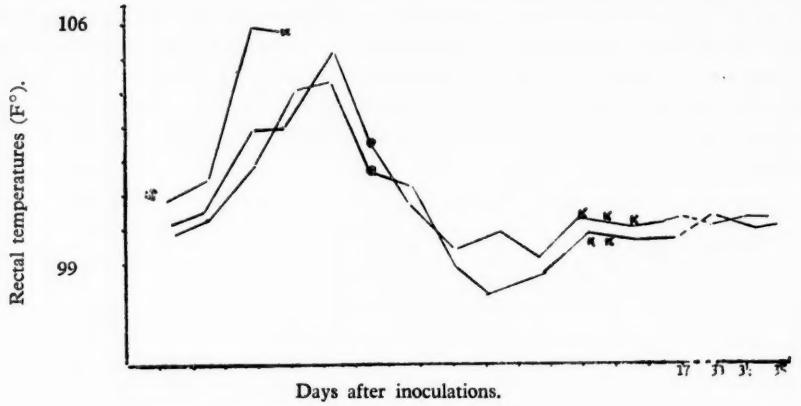

FIG. 6

Intraperitoneal inoculations of three dogs with purified virus.
Blood in faeces ●; Kerato-conjunctivitis K; Death X.

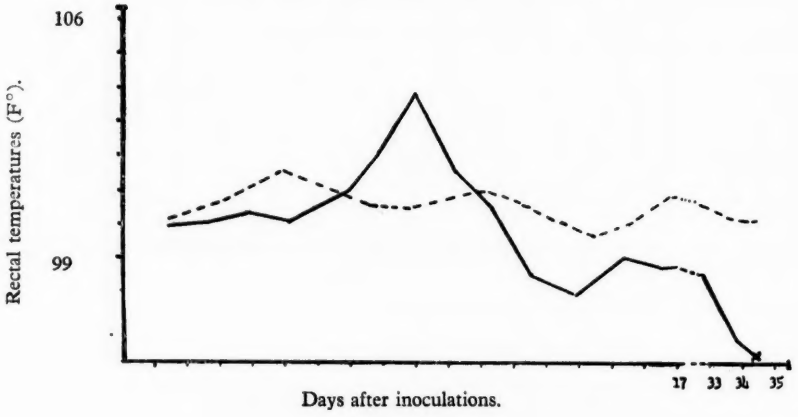

FIG. 7

Intraperitoneal inoculations of two dogs with heated virus.
56° for 30 minutes ——; 100° for 30 minutes - - - -; Death X.

of active virus from time to time has imposed new amounts of the virus antigen upon the antibody forming system and thereby permitted the booster effect to operate.

Virus Characters

Although it is known that the frozen virus survives for long periods (Larin, 1951a), the stability of the virus in the freeze-dried state is remarkable. In 1950 we received

from Professor Rubarth of Stockholm a few hermetically sealed vials (penicillin type) containing a strain of the virus in the form of freeze-dried liver. All the vials but one were used for experiments, the latter being left on a laboratory shelf at room temperature (the maximum being 22°-25°C.) and exposed to light. Six years later the virus in the vial was tested for virulence to dogs. All three dogs which were inoculated intraperitoneally with the virus, developed acute virus hepatitis, and one of them ultimately died of the disease. Apart from this experiment we have also studied the resistance of the virus to different agents. These studies were conducted with the use of purified virus preparations. A study of the resistance of the virus to heating is illustrated by typical protocols shown graphically in Fig. 6 and 7. From Fig. 6 it can be seen that non-heated virus aliquot produced a severe disease in three dogs inoculated intraperitoneally, one case proving fatal ultimately. The other two aliquots of the virus batch were heated, one at 56°C., and the second at 100°C. for 30 mins. It can be seen from Fig. 7 that the aliquot which was heated at 56°C. produced fatal disease in the dog inoculated intraperitoneally, but the duration of illness in this case was longer than that produced by non-heated virus (Fig. 6). A dog inoculated with virus which had been heated at a 100°C. for 30 mins. remained normal on inspection, but three weeks after inoculation it developed a good titre of the complement fixing antibody, this showing that after heating for 30 mins. at 100°C. the virus was antigenically active, although it was not known whether it multiplied or not. Experiments in which the virus was subjected to ultra-violet irradiation are shown graphically in Fig. 8. Aqueous suspensions of purified virus were irradiated for two hours at room tempearature in "Vycor" glass tubes sealed with rubber stoppers. The tubes were placed horizontally at a distance of 15 cm. from a 15W. mercury vapour lamp ("Hanovia") emitting predominantly at 2537A°. The irradiation was controlled by varying the times of exposure. Virus aliquots in "Vycor" tubes protected from irradiation with tinfoil served as controls. The irradiated and control virus were inoculated into dogs subcutaneously. The results in Fig. 8 show that although the virus was obviously affected by U.V. irradiation the total destruction of infectivity was not achieved by two hour irradiation. The U.V. irradiated virus (a) protected dogs against inoculations with the unexposed virus; (b) gave rise to the antibody in inoculated dogs; and (c) was antigenically active in the complement fixation reaction. Antigenicity of the virus in vitro and in vivo were also not affected by killing it with 0.1% formaldehyde.

Discussion

Discussing the mechanism of convalescent immunity in canine virus hepatitis and how it may be simulated by the use of a vaccine, it is important to bear in mind the following features of Rubarth's disease which have been noted in this paper.

1. The multiplication of canine hepatitis virus in the dog does not necessarily result in apparent illness. Clinical signs of illness are not evident in the majority of affected dogs, whereas in the others it appears mainly in a mild form. The virus behaviour in the dog is illustrated in Fig. 2 from which it can be concluded that the virus belongs to the group of viruses which are naturally relatively avirulent and which give rise to latent infections in the majority of infected subjects, with clinical disease being the unusual occurrence in a few. Therefore latent infections may be looked upon as a more common source of infection than is generally supposed.

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2. Reflecting upon the epidemiology of canine virus hepatitis and of its associated immunity it would seem to be supported well by experimental evidence that the virus can persist and multiply in immune dogs for long periods, and thus it can be said that whilst such dogs possess immunity to " disease ", they are obviously not immune to " infection ".

3. To recapitulate the characters of the virus, it is important to emphasise the high resistance of the virus to physical agents, and also that virus is immunologically stable (Larin, 1951a, 1958b).

In considering the matter of artificial immunisation against canine virus hepatitis, it is important to distinguish the following possible approaches to this problem from a practical standpoint.

The purpose of vaccination.

It is obvious that those possessed by a zeal for vaccination against the fatal form of the disease will find no difficulties for this form is rare under the natural conditions and it is not reproduced so easily experimentally even if the virus is injected by the intravenous or intraperitoneal route. Hence if the virus is administered by a more

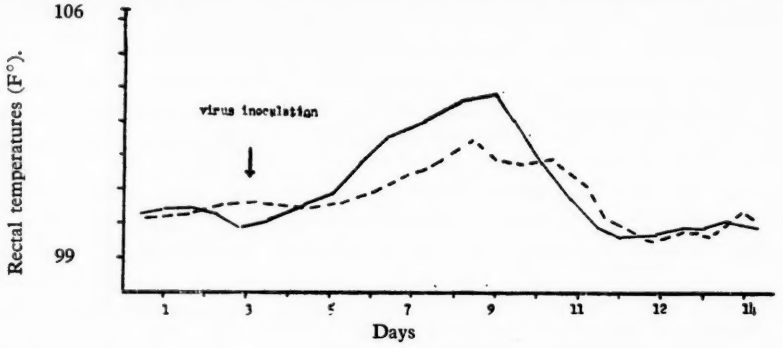

FIG. 8
Inoculations of dogs with irradiated virus.
Virus control ——; irradiated virus - - - -.

natural route there is little risk of the virus inoculated dogs reacting severely. This fact has been shown by Mansi (1953) who studied conjuctival instillations of the living virus to susceptible dogs, and Mansi's results found full confirmation in experiments reported in this paper. Thus the hazard of using the naturally occurring virus for the active immunisation is small because Nature has already done a good job of " attenuating " this virus without the help of laboratories.

The second approach to the active immunisation would be to control the subclinical infection and thus to prevent the development of virus lesions in the vital organs. In previous papers evidence has been presented to show that the presence of virus lesions can aggravate superimposed infection of either viral or even bacterial nature (Larin, 1953; Laughton and Larin, 1954; Larin and Orbell, 1956).

The use of a living vaccine.

If one were to consider the use of a living vaccine, then one would be increasing artificially the chances for contracting infection by susceptible dogs, if it is assumed

that the virus in the vaccines would spread from dog to dog. Hence a question that will have to be answered is whether or not a living virus vaccine would not set up in vaccinated dogs a latent infection which in all essentials would be similar to the naturally occurring latent infection.

The use of a killed virus.

Another way of simulating convalescent immunity is by the use of a killed virus. The general principles of immunisation with a killed virus vaccine require that there must be (1) a suitable method for destroying the infectivity but not the antigenicity of the virus, (2) a sufficient antigenic mass in the immunizing dose of the vaccine, and (3) the availability of the virus in a relatively pure form, so as to avoid the interfering effect of the extraneous antigens.

This study provides an indication as to what might be expected if a killed vaccine would alter the immunological mechanism so that even if the persistence of antibody is not demonstrated following vaccination, the primary stimulation with the vaccine may act as a conditioning stimulus. The "anamnestic" reaction which is associated with booster infections, has been shown in Fig. 1 and 4.

Sasaki *et al* (1956) demonstrated also that the specific stimulation can be achieved with the use of a killed virus preparation.

Hence it would appear that a killed virus preparation can be a simple and safe means for the primary stimulation which might be expected to be of great value later on exposure to natural infection.

Summary

The purpose of this paper was to describe the results of a study of the mechanism of convalescent immunity in canine virus hepatitis and to derive therefrom clues as to how this infection may be controlled by artificial immunisation. The mechanism whereby convalescent immunity may be simulated by a vaccine has been discussed in detail. The proper adjustment of dosage of the purified virus which has been rendered non-infectious without the loss of the antigenic powers, may provide the safe means for active immunisation against canine virus hepatitis.

Acknowledgment

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THE SIGNIFICANCE OF CYSTIC ENLARGEMENT IN THE BOVINE CORPUS LUTEUM

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Laszlo (1937) encountered an unspecified incidence of cystic corpora lutea in a series of dissections involving 300 sets of cow genitalia. He was careful to differentiate between these structures and luteal cysts, in which no ovulation had taken place, and assumed tentatively, that a central hollow of up to 1 cm. diameter occurred in a proportion of normal corpora, while any larger hollow he regarded as pathological. Wright (1945) confirmed that a small central cavity persisted in approximately 25 per cent. of normal dioestrous luteal bodies. As blood clot or fibrin threads occurred in the cystic fluid, Laszlo ascribed the cystic formation in many cases to over-rapid sealing of the follicle after ovulation. Smythe (1942, 1943), stated that a central hollow in the corpus as small as a wheat grain could be pathological; he claimed to have followed clinically the development of such cystic bodies from apparently normal corpora, at about the time these were due to regress, and noted also associated uterine abnormalities.

Ajello (1947) supported Laszlo's opinion that the cystic corpus luteum could be a normal structure and found one in a pregnant cow; but in a series of 12 cases in which the cows' uteri also were sectioned, there was evidence of infertility in at least 4, with a bias towards the form of (follicular) cystic ovarian disease, the most consistent infertility feature consisting of the characteristic associated uterine glandular hyperplasia. Hancock (1948), after a clinical study, agreed with Smythe that the cystic corpus was associated with suppression of oestrus, and with Ajello that there was some sort of connection with follicular cyst formation. Nevertheless he observed cystic corpora enlarged up to golfball size in three pregnant animals; on rupture these bodies were resolved to normal, and the normal course of pregnancy remained unaffected. Asdell *et al.* (1949) found up to 1.6 ml. fluid in 4 of 18 heifer luteal bodies and hence denied that a cystic corpus could be pathological. Hammond (1927) claimed that it was a normal stage in development.

The existing literature thus appeared to be lacking in quantitative data on the incidence, both absolute and relative to one another, of both the normal and pathological types of cystic corpora, on the existence of which most authors were agreed. Clinical studies had been carried out on small or unspecified numbers of animals but they were not checked by examination *post-mortem* in all cases. Laszlo's broadly-based investigation after death lacked any form of clinical control.

Introduction

In the course of an investigation on 274 barrener discard cows and a proportion of reproductively normal controls, which involved a routine clinical study followed by post-mortem examination, the opportunity occurred to try to fill the gaps in existing knowledge, which a review of the literature revealed. Evidence of cystic corpus formation was found in 41 animals, the clinical studies on which averaged 50 days

in length; adequate histories, including status as regards venereal etc. infection were available, and as well as the ovaries, the associated uteri and oviducts were studied by dissection, bacteriological culture, and H and E-stained microscopic sections.

Fourteen of the cases were provisionally classed as "borderline", in that there was some doubt, both as to whether the cystic condition, which was to some extent observed in the corpora, was in fact characteristic of these bodies in the fully mature state; and on whether the hollow condition of the organ was associated with a genuine diminution of luteal tissue below the average volume. In view of the great variation

TABLE I

Examples of Case Histories.

Borderline Cystic Corpus Luteum Cases:				
Cow	Group	Clinical History of C.l.	Autopsy C.l. % luteal tissue	Autopsy C.l. ? Time to mature
101	Adrenal Virilism Normal Uterus	C.l. clinically diagnosed as cystic but not followed beyond 6th day.	No sign of hollow in remnant. 85%. C.l. 2.5 cm.	
287		C.l. clinically diagnosed as cystic. Not ruptured.	hollow 0.75, contains jelly.	7 days old.
286	Severe Endometritis	C.l. not diagnosed as cystic and no previous history.	75%. C.l. 2.5, cavity 0.8 with fibrous clot.	About 8 days old.

Definite Cystic Corpus Luteum Cases:				
Cow	Group	Clinical History of C.l.	Autopsy C.l. % luteal tissue	Autopsy C.l. ? Time to mature
217	Adrenal Virilism	Ruptured, 2 days premortem, 6 days from ovulation (and reformed still cystic).	20%. C.l. 2.75, with hollow 2 cm. diameter.	Yes, 8 days from ovulation.
160	Normal Uteri	Very hollow but accompanied normal C.l. Onset of next heat delayed till 28 days after last.	10%. C.l. 1.25 cm., thin walls.	Not normalised at autopsy, 10 days after rupture.
305	Normal Uteri	C.l. very obviously cystic, 3.0 cm., and "exploded" on contact. 11 days so definitely abnormal.	70% rupture the previous day probably having some 'normalising" effect.	Not applicable.
181	Severe Endometritis	Full-grown C.l. not clinically cystic though earlier it did so feel. 4 serial examinations.	80%. C.l. 2.25 cm., hollow 1.0 cm. diameter.	Yes, 9 days old.
134	Bilateral Bursitis	C.l. not clinically diagnosed as cystic.	25%. C.l. 3.0 cm.	12 days old.

in weight of normal corpora reported e.g. by Foley and Reece (1953), nothing beyond the estimate by eye of the percentage of normal tissue volume (in relation to external diameter) was considered to be worth attempting. Cavity diameters were measured and the ages from the last heat were checked by clinical findings and usually by the bulls service register. The results of analysis of the borderline series, as exemplified by cases 1-3 in Table I, gave a mean percentage of normal tissue volume as high as 70, a mean cavity diameter of 8 mm. and an average age from last oestrus of dissected

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corpora of only 6 days. The age of only one corpus exceeded 8 days, a significant finding consistent with the idea, anticipated in the paper by Laszlo, that a proportion of corpora may in the course of normal development remain demonstrably hollow until the attainment of their maximum tissue volume, at about 8 days from oestrus (Asdell, 1949). Thus broadly speaking the indication from these 14 cases is that the corpora concerned represent a normal stage in the growth of a naturally-occurring proportion of healthy bovine luteal bodies. The mean cavity diameter at 8 mm., approximates closely to Laszlo's figure of 1 cm., which he adopted as an arbitrary demarcation between normal and pathologically hollow corpora.

Results

The contrasting results from the remaining 27 cases emphasise the importance and interest of this " definitely " cystic series of corpora. In 17 of these, developed corpora were still present at autopsy and they had a mean estimated tissue volume percentage of only 37, a cavity diameter of 1.6 cm. and a mean age since last oestrus

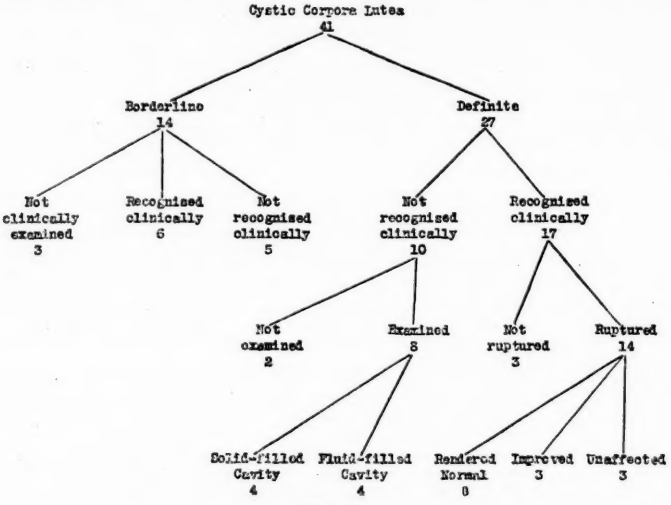


FIG. I
Schematic Classification of Cystic Corpora Lutea.

of 13.5 days (2 were 6 days old, the remainder 9 days or over). External diameter averaged 2.7 cm., only a little greater than that either of a normal solid corpus at about 2.3 or of the borderline series, (which at 2.5 suggests that the hollow formation here more than counterbalances any immaturity). Thus there is ample evidence of the marked subnormal quantity of luteal tissue present, in the definitely cystic corpora, at an age when they should have long reached their maximum growth. Calculation gives the mean cavity volume of the " definite " series as 9 times greater than that of the " borderline ", using the measured diameters of 16 and 8 mm. respectively!

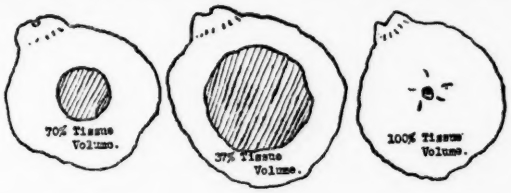
In 10 of the " definite " cases the abnormal corpus luteum was recognised only on dissection of the ovary; in 8 of the 10 the corpus had undergone clinical examination and in 4 of these 8 there was some apparent reason for the failure to reach a

positive clinical diagnosis. In two instances the cavity was filled with solid or organising bloodclot rather than cystic fluid, one body had an 80 per cent. tissue volume and the cavity was rather small, and the fourth lay adjacent to two solid corpora lutea in the same ovary. In the other 17 cases the cystic condition of the body was diagnosed clinically, a finding confirmed in 11 at examination post-mortem. The lack of such confirmation in the remainder cannot be considered as evidence of the inaccuracy of manual diagnosis per rectum (see below). It appears that a clinical positive diagnosis of cystic corpus is highly accurate, 25-35 per cent. of corpora may remain undetected clinically, but some of them on account of their firm consistence due to clotting etc.

In 3 of these 17 cases an obvious cystic corpus was left in situ. One of these felt cystic 4 days after ovulation but was normal in 6 days and was found on autopsy at 8 days with an 80 per cent. tissue volume and 10 mm. cavity. This sequence must approach closely to the normal maturation process, but in the other two cases with intact clinically recognisable cystic corpora (cows 95 and 78) there was clear evidence of a pathological involvement.

Effect of Intervention

In the remaining 14 cows the cystic corpus was ruptured in the course of the clinical examination. This was done deliberately in only 4 animals, since in the other 10 the bodies proved too fragile to withstand gentle palpation, though usually it was possible to establish the size and the presence of an ovulation scar before rupture took



Borderline Definite Normal

FIG. II

Mean of Types of Cystic and Normal Corpora Lutea drawn to scale (Natural size x 1).

place. In 7 of the 14 this occurred at from 4-7 days after ovulation, the development of the cystic corpus being followed at 48-hourly intervals in 4 cows from the preceding heat. In one the age of the cystic body from the last ovulation was unknown, but in the last 6 the age from last ovulation varied from 9 to 21 days with a mean of 13 days, so that the pathological nature of the corpus would seem to be established beyond doubt. The bodies 21 and 11 days old at rupture were each traced in development from the ovulation stage, in the course of 5 serial examinations. In three of the 14 cows with ruptured cystic corpora, the bodies were affected but little by the procedure, and reformed, and none was estimated at more than 25 per cent. tissue volume on autopsy, although slaughter was delayed for a mean of 7 days after operation. In one of these three cases rupture had occurred only 6 days from ovulation; but the persistence to autopsy of so cystic a corpus provides additional evidence that this cow's condition was in fact pathological.

Rupture of the cystic corpus appeared to have a " curative " effect on the condition in 11 of the 14 subjects. This effect was total in as many as 8; in all of which a

clinically normal corpus luteum appeared to be the result of rupture. In two cases a seemingly normal corpus was already established at 2 and 3 days, respectively, after the manipulation, while one of the others was not re-examined before slaughter and the remainder only at 5, 6, 11, 9 and 15 days (when normal corpora were present). Three of the 8 were slaughtered before regression of these corpora became due, and in each a normal, completely solid corpus was found upon dissection of the ovary. One slaughtered a little later in the cycle yielded a solid regressor of 1.5 cm., while two yielded normal solid remnants after a subsequent heat had occurred. One of these cows had been subjected to rupture as late as 11 days after the ovulation which produced the cystic corpus. In the last two (operated on 21 and 9 days after heat) these remnants remained conspicuously hollow, confirming the original clinical diagnosis but suggesting that the cystic condition had survived rupture, although this had resulted in a noticeable luteal tissue increase. These observations on the effect of rupture recall Hancock's finding that he thus "normalised" cystic corpora in 3 pregnant cows which successfully carried to term.

In 3 cases the curative effect of rupture was incomplete at the time of slaughter; but as in 2, this took place as early as 24 and 48 hours afterwards, evidence of a very rapid improvement was obtained, both corpora at autopsy having a small cavity and an estimated 70 per cent of normal tissue volume, although in each case the originally cystic corpus had collapsed "explosively" on gentle exploratory palpation. The last case killed 8 days after rupture yielded a body with at least 85 per cent. normal tissue volume but a central cavity 8 mm. in diameter. At the sixth day this body was normal by the standards of clinical examination.

Suppression of Oestrus

Smythe's claim (supported also by Hancock) to have observed some persistence of the corpus luteum as it became cystic, with resultant lengthening of the interoestrous interval, was substantiated by the findings from as many as 4 of the cases. The best-established case was observed over 11 serial examinations. The cystic nature of the corpus luteum was apparent by the sixth day after heat and continued little altered until rupture on the 21st day. On 23rd and 24th day the corpus was normal on clinical examination, but regression had begun by the 26th day and was complete when oestrus returned on the 29th day from the previous heat. In the two cows, numbers 95 and 78, receiving 6 and 5 serial examinations, a cystic corpus was fully developed at the first examination; in one it persisted unaltered until autopsy 25 days later; while in the other a very slow regression occurred over 23 days, at which time oestrus returned. The luteal remnant was notably hollow on dissection. In the fourth of these cases, receiving 6 serial examinations, an interoestrous interval was prolonged to 28 days when 19 days had been usual for this cow; at one examination the mature corpus luteum felt cystic, and the subsequent one, upon slaughter 10 days after the final heat, proved to be so on dissection. Three other cases gave definite evidence of *repeated* cystic corpus production. In one 2 consecutive bodies underwent rupture; the others, each seemed to have cystic corpora at early clinical examinations—one was ruptured—and six weeks later yielded others from the opposite ovary. In one of these cows the "intervening" corpus was found clinically normal 25 days before slaughter. For the other purposes of this paper, only the data from the cystic corpora actually

dissected from these 4 animals have been incorporated. In a fifth and a sixth cow, the luteal bodies preceding the cystic one which was dissected had normal consistency but abnormally short lives of only 6 and 8 days as compared with 16.

Hancock stated, largely on clinical examination results, that the characteristic cystic corpus luteum "often shows no evidence of an ovulation point". If this idea were to be confirmed the question of differentiation from the unovulated luteal cyst would offer immediate difficulties. In the present series of 41 cystic corpora, of which

TABLE II

Group Incidence of Cystic Corpora Lutea.

Group	Group Total Cases		No. Definite Cystic C.1 Cases		% Definite Cystic C.1 Cases		No. Borderline Cystic C.1 Cases		% Definite plus Borderline Cases	
1. Normal Control.	12		1		8		1		16	
2. Simple Nymphomania.	33		1		3		—		3	
3. Cyclic Nymphomania	19		2		10		2		20	
		27		4		15				
4. Adrenal Virilism.	8		2		25		1		37.5	
5. Normal Uteri.	33		4		12		4		24	
6. Severe Endometritis.	59		6		10		4		17	
				6		6		4		10
7. Endosalpingitis, (mostly plus severe Endometritis).	40		—		—		—		—	
	28		5		18		—		18	
8. Unilateral Bursitis.		46		10		22				22
9. Bilateral Bursitis.	18		5		28		—		28	
10. Encapsular Bursitis, Unilateral.	9		—		—		—		—	
		26		1		4				12
11. Encapsular Bursitis, Bilateral.	17		1		6.5		2		19.5	
12. Not Sectioned.	10		—		—		—		—	
TOTALS	286		27		9		14		14	

33 were actually dissected, a normal, full-size, readily recognisable ovulation scar was present in 38 and in the remaining 3 an ovulation "point" or "pimple" was obvious when the ovaries were removed from the cow, although this structure could not have been recognised per rectum with certainty.

Correlation with Other Genital Tract Lesions

The 286 cows used in the study were grouped basically according to the condition of the uterus and oviducts; in this scale, ranging from "normal uteri" to "bilateral encapsular bursitis", the greater degree of involvement can as a rule be assumed to include the lesser. The evidence of abnormality in the normal uterus group affected mainly the ovaries and this was more strikingly the case in the 3 special groups comprising cases of true cystic ovarian disease involving impairment of the ovulation function. Uterine and tubal involvement was common in these groups. Table II shows the distribution of cystic corpus luteum cases according to these groups. The Normal Control Group I is still too small to justify comment on its incidence of cystic corpus cases, beyond its confirmation that this condition need not be abnormal. The

low incidence in the simple nymphomania group is to be expected, as the luteinising function is largely suppressed in this form of disease, while in cyclic nymphomania and adrenal virilism the nature of the endocrine disease, would in theory tend to promote cystic corpus luteum formation, an expectation borne out by the results obtained in the field. Thus for instance incipient degeneration of the two follicular thecae might, in cyclic nymphomania, impair the ability of these cells to luteinise in response to the pituitary stimulus. More advanced thecal degeneration of some follicles, in both cyclic and simple nymphomania, characteristically prevents ovulation, although solid non-ovulated luteal bodies are sometimes formed as well as large follicular cysts. Again in the normal uterus Group 5 the high incidence of cystic corpora as an evidence of disturbed function is to be expected (see above). In Groups 6 and 7, where the chief characteristic is the result of ascending infection which has not reached the abdominal end of the oviduct and which has resolved, though with some permanent replacement of functional tissue by fibre, the incidence of cystic corpus luteum should be lower than in Groups 3-5 inclusive, and might be held comparable, in theory, with that of a normal control group of similar size. The table shows that the incidence is in fact lower in the endometritis group 6 than it is in groups 3-5, while group 7 includes no cystic corpus luteum cases at all. At the present stage it seems justifiable to ascribe the difference between groups 6 and 7 to chance and to consider them jointly as a control group for the others. The low incidence in the encapsular groups, is accounted for readily by the observation, that in most of these cases the mechanical pressure of the fibrous capsule interferes with the ability to ovulate. Only occasionally is a portion of ovarian surface left sufficiently exposed for the normal luteinising process to occur. Table II also reveals that the proportion of " borderline cystic " corpora, shows a group-by-group incidence well in step with that of the definitely cystic corpora, a finding which is significant. The two cases in the " borderline cases " most nearly conforming to " definite " requirements occurred in the high incidence groups 3 and 5; one of these was in a state to be ruptured 8 days after ovulation, and the other had a lowered tissue volume and showed few regression signs after 21 days.

Relationship with Bursitis

It remains to account for the high incidence of cystic corpus occurring in the unilateral and bilateral simple bursitis groups 8 and 9. There seemed no obvious reason in the first instance why the incidence should be any higher in these groups than in groups 6 and 7 where the general pathology was fundamentally similar, and as here the confirmation from the occurrence of the borderline type cases was lacking, a more detailed analysis of the individual histories was undertaken, in the attempt to determine just how significant the apparent high incidence in these groups might be. The features associated with the furthest and most injurious deviations from the normal state in cystic corpora lutea are listed on the left-hand side of Table III, and the next column shows the number of cows in the definite series to exhibit each feature. The remaining columns show how that total is distributed as between the cystic ovarian disease and normal uterus groups (Category I), the endometritis and endosalpingitis groups (Category II) and the bursitis group (Category III). The normal control and encapsular cases (1 of each) are excluded. The totals in column 3, 4 and 5 indicate strikingly, even when corrected for the differing numbers of cases in the categories,

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that the type of cystic corpus encountered in category II differs relatively little from a normal functional body. This is a result in line with the view already expressed, that the incidence in category II might be of a "normal control" type. The figures of this table also provide evidence, however, that the cystic corpora produced by the simple bursitis cases of category III are not much less pathological in nature than those of category I where the ovaries are the organs primarily diseased. There is in

TABLE III

Severity of Involvement by Groups.

Column 1 Features associated with most injurious deviation from normal in the cystic corpus luteum.	Column 2 Number of Cows Positive for Feature	Column 3 Number in Category I (9 animals)	Column 4 Number in Category II (6 animals)	Column 5 Number in Category III (10 animals)
Poor response to rupture.	3	3	—	—
Persistence of corpus with suppression of oestrus.	2	1	—	1
Long interval (8 days or over) from last ovulation.	9	3	2	4
Lowest tissue volume: 25% or under	5	3	—	2
Tendency to repeat cystic formation in successive cycles.	6	3	—	3
Poor development of ovulation scar.	3	1	1	1
Column Totals		14	3	11
Relative column values ... ... (Sum of Columns 3-5 figures) / (expressed as % Category totals) / (10)		15.4	4.95	11

fact a strong suggestion that the presence of bursitis is a significant factor. This view receives further support from the finding that these more pathological features are comparably well distributed in categories I and III, seven cows in each showing at least one feature, or 78 and 70 per cent. of the categories respectively, whereas in category II only 2 cows, 33 per cent., show any of the features. And further 4 bursitis cows of category III each exhibit 2 of the features; the column 5 score of 11 being completed by another 3 showing a single feature each. In category I each of four animals show only one feature, one showing 2, one 3, and one as many as 5 separate features: the score by this category is higher not because more animals are involved, but because they are involved more deeply, as is to be expected in these, primarily ovarian disease groups. Such results do seem to suggest that the ascension of infection (*C. pyogenes* mainly) beyond the abdominal ostium of the oviduct can (in not more than about 20 per cent. of cases) interfere sufficiently with the ovulation and luteinising processes to produce a rather markedly abnormal type of cystic corpus luteum. This is a sufficiently remarkable conclusion, in view of the fact that a vast volume of recent research is unilaterally against the old theory of a comparable origin for cystic ovarian disease proper. Further work in confirmation is most necessary, but it is interesting that in four of the 5 unilateral bursitis cases the cystic corpus and the bursitis occurred on the same side of the tract, while in the fifth case where they were on opposite sides there was a more severe endosalpingitis on the cystic side than on that carrying the adhesions.

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Discussion

In 286 cows, of which 274 were discarded for failure to breed, 27 definite cases of abnormally cystic corpora lutea were encountered, characterised by an average tissue volume estimated at only 37 per cent. of normal, a mean internal cavity diameter of 1.6 cm. and a mean age since the last oestrus of 13.5 days. 25 of these corpora were examined clinically and the four with solid contents—bloodclot etc.—were not detected in this manner, neither were 4 others; but no false positive diagnosis was made. In 14 cases the cystic corpora were ruptured; 3 reformed unaffected but 8 were rendered quite normal in appearance at post-mortem examination and 3 were improved. Considered in relation to the lesions elsewhere in the cows' genital tracts, the highest incidence of the corpora most obviously abnormal was found, as expected, in the groups with cystic ovarian disease *sensu stricto,* or other purely ovarian disturbances. But a comparable incidence and degree of severity occurred in the simple bursitis series; in unilateral cases the cystic corpora always occurred on the affected side, suggesting unexpectedly that ascending infection can in such cases interfere with the normal process of luteinisation.

In addition to these 27 cases, 14 positive cases of cystic corpora lutea were met with, in which it remained possible that the formation seen was consistent with a form of the normal maturation process; the internal cavity diameter averaged 8 mm., tissue volume 70 per cent. and age from last heat only 6 days. (Single clinical observations or autopsies from a further 28 cases supported such a hypothesis of cystic type development to the eighth day.) Besides these, however, a low incidence, included among the 27 more abnormal cases, occurred in normal control animals, and disease groups where ovarian pathology might be expected to be minimal. This indicates that possibly up to 10 per cent. of animals with normal ovaries may produce some corpora apparently quite abnormal and subfunctional by the criteria adopted.

These results suggest that no harm is likely to result in any circumstances from the rupture, deliberate or inadvertent, of a cystic corpus lutem. If this body is in existence more than 8 days from the last observed heat, or *a fortiori* if there should be suppression of oestrus and a tendency to persistence, as described by Smythe (1942) and shown by 4 of the present cases, the cystic condition of the corpus provides evidence of the existence of a functional disorder, which the intervention will probably set right. No case was encountered of a " simulated persistence " in which a suboestrus was followed by the development of a fresh initially-cystic corpus which attained a normal consistency by the ninth day, but it seems very likely that such cases do sometimes develop in the field. Both Smythe and Hancock wrote of cystic corpus luteum as occurring in Guernseys, but of the 27 most-affected cows in the present study, although selected from 166 Guernseys and only 120 other cattle, only 16 were Guernseys while 5 were Ayrshires, 4 Friesians and 2 Jerseys, roughly in equal proportion to the total number of each breed in the sample as a whole.

Summary

In 286 cows there were 27 definite cases of cystic corpora lutea. Seventeen of these were detected by clinical examination and in 14 cases the cysts were ruptured. In a further 14 cases, the cystic corpora were consistent with a form of the normal maturation process. The results suggest that no harm is likely to result from the rupture of cystic corpora lutea. In certain cases good effects may be expected.

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THE ANTICOCCIDIAL ACTIVITY OF GLYCARBYLAMIDE

by

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The anticoccidial agent 4, 4′ - dinitro - carbanalide - 2 - hydroxy - 4, 6 dimethyl pyrimidine (nicarbazin) is of value as a prophylactic in controlling caecal (*Eimeria tenella*) and intestinal (*E. necatrix*) coccidiosis in fowls, (Cuckler, Malanga and Ott, 1956). This product, however, when fed to laying birds may cause abnormalities in the eggs, (Baker, Hill, van Tienhoven, and Bruckner, 1956; Lucas, 1958).

The recent introduction of 4, 5-imidazole dicarboxamide (glycarbylamide) has provided a new anticoccidial drug which is claimed to have none of the disadvantages associated with nicarbazin when used prophylactically over long periods. The present paper reports the results obtained in a series of tests of the anticoccidial properties of glycarbylamide against diseases caused by *E. tenella, E. necatrix,* and *E. acervulina* in the fowl and by *E. meleagrimitis* in the turkey. No toxicity or drug-resistance studies have yet been made.

Materials and Methods

Rhode Island Red (RIR), White Leghorn (WL), and RIR x WL chickens, and Broad-breasted bronze turkey poults, aged from 7 to 24 days, were used in these tests. The chickens were run on wire floors throughout. The infective material consisted of oocysts of *E. tenella, E. necatrix, E. acervulina* and *E. meleagrimitis* obtained from single cells administered to coccidia-free chickens or turkey poults kept in isolation units. The age of the oocyst-suspensions used never exceeded 5 weeks.

The chicken ration was that normally used at this Station (see Horton-Smith and Long, 1956). The glycarbylamide was supplied as a 25 per cent. premix which was incorporated in the food at the required concentrations by mixing for 20-30 minutes in an electrically-driven Hobart mixer.

Experimental Results

A. E. tenella and *E. necatrix.*

1. The effect of starting medication before infection.

Experiment 1. Six groups of six or seven 23 day-old chickens were treated as follows: Groups 1 and 2 received 0.003 per cent. w/w glycarbylamide in the food; groups 3

and 4 received 0.0125 per cent. w/w nicarbazin in the food; groups 5 and 6 received non-medicated food. Twenty four hours after medication was started in groups 1-4 the chickens of groups 1, 3 and 5 were each given approximately 100,000 sporulated oocysts of *E. tenella*. The chickens of groups 2, 4 and 6 each received similar numbers of oocysts of *E. necatrix*. Groups 1-4 were given the medicated food for ten days in all. Surviving chickens of all groups were destroyed on the eighteenth day after receiving the oocysts.

No deaths occurred in groups 1-4. The six chickens in group 5 all died from acute caecal coccidiosis and six of the seven chickens of group 6 died from the intestinal disease. In this experiment glycarbylamide gave the same protection as nicarbazin though used at quarter the concentration.

Experiment 2. Five groups, each of ten 18 day-old chickens, received food which had been medicated with 0.003 per cent. w/w glycarbylamide. Forty eight hours after medication had started each chicken was infected with 100,000 sporulated oocysts of *E. tenella*. Medication was continued for 3 days (group 1), 4 days (group 2), 5 days (group 3) 6 days (group 4), and 7 days (group 5). These differences in duration of

TABLE 1

The effect of feeding 0.003 *per cent. glycarbylamide before infecting with oocysts of* E. tenella *and for different lengths of time afterwards.*

Group	Deaths from Caecal Coccidiosis: Days relative to infection with 100,000 occysts														Total
	−2	−1	0	+1	+2	+3	+4	+5	+6	+7	+8	+9	+10	+11	+12	Mortality
1	S			T				5	1	1						7/10
2	S				T				5		1					6/10
3	S					T					1					1/10
4	S						T									0/10
5	S							T								0/10
6	No treatment							6	2	1						9/10

S = Start of treatment.
T = End of treatment.

medication were adopted to give an indication of the phases of the coccidial life-cycle most affected by the drug. The chickens in a sixth group were each infected with 100,000 sporulated oocysts but received no medication. The results of this test are summarised in Table 1.

A substantial number of deaths occurred in groups 1 and 2 in which treatment ended on the first and second days of the infection respectively. The majority of deaths in group 1 occurred promptly on the fifth day of the infection. Most deaths occurred on the sixth day in group 2 which suggests some activity against early schizogony. Few deaths occurred when medication was stopped on the third day of infection (group 3), and protection was complete when medication was extended to the fourth and fifth days of infection. Although some effect was exerted against the first schizogony stage, the second stage was the more susceptible to the action of the drug.

Experiment 3. Two concentrations of glycarbylamide were used in the food and medication was started twenty four hours before each chicken of each group received 100,000 sporulated oocysts of *E. necatrix*. Medication with each concentration was continued for four to ten days.

Six groups, each of eight 16 day-old chickens, were treated as follows: Groups 1 and 2 were given 0.003 per cent. w/w glycarbylamide in the food and groups 3 and 4 received 0.005 per cent. w/w glycarbylamide in the food; groups 2 and 4 had four days' treatment and groups 1 and 3 ten days' treatment. Group 5 received no medication but was also infected with 100,000 sporulated oocysts. A sixth group was kept on unmedicated food and was not infected until later; this group served as a control to a challenge dose of oocysts which was administered to the survivors in the medicated groups to test their resistance to a second infection. The results obtained in the first part of the experiment are given in Table 2.

TABLE 2

The effect of feeding two concentrations of glycarbylamide for different lengths of time starting from the day before infecting with oocysts of E. necatrix.

Concentration of glycarbylamide in food	Deaths from intestinal coccidiosis: Days relative to infection with 100,000 oocysts												Number of chickens	Total deaths from intestinal coccidiosis
	−1	0	+1	+2	+3	+4	+5	+6	+7	+8	+9	+10		
0.003%	S										T		8	0
„	S				T					2	2		8	4
0.005%	S										T		8	0
„	S				T					3	1		8	4
None							2	4	1	1			8	8

S = Start of treatment.
T = End of treatment.

Fourteen days after the first doses of oocysts had been administered, challenge doses of 200,000 sporulated oocysts of *E. necatrix* were given to each of the survivors from groups 1-4 and to each chicken of the sixth group, which had not previously been infected. One survivor from group 1 died from intestinal coccidiosis on the seventh day. No deaths occurred in groups 2, 3 and 4, but seven of the eight control (group 6) chickens died on the fifth day and the remaining one on the seventh day.

Although there is some control of intestinal coccidiosis caused by *E. necatrix* when medication with glycarbylamide is withdrawn on the third day of the infection, better control is obtained when treatment covers the period of late schizogony. Medication does not interfere with the development of immunity to this form of coccidiosis. Little difference was observed in the results obtained by using either of the two concentrations of drug over the same periods of time.

Experiment 4.

This experiment was performed to ascertain the minimum length of time for which medication must be continued to give greatest protection against disease produced by administering a single dose of oocysts of *E. necatrix,* and also to determine which

phases of the life-cycle of *E. necatrix* were most susceptible to the action of glycarbylamide.

Fifty four 13 day-old chickens were distributed among six groups, each of nine chickens. Groups 1-5 were given access to 0.003 per cent. w/w glycarbylamide in the food for 3, 4, 5, 6 and 7 days respectively. Two days after the start of medication each chicken in these groups and a sixth group on non-medicated food was given 100,000 sporulated oocysts of *E. necatrix*. The results of the experiment are shown in Table 3.

TABLE 3

The effect of feeding one concentration of glycarbylamide for different lengths of time starting two days before infecting with oocysts of E. necatrix.

Concentration of glycarbylamide in food		Deaths from intestinal coccidiosis: Days relative to infection with oocysts												Number of chickens	Total deaths from intestinal coccidiosis
		−2	−1	0	+1	+2	+3	+4	+5	+6	+7	+8	+9	+10	
0.003%	S				T				2	3	3				9	8
„	S					T					5		4		9	9
„	S						T						3		9	3
„	S							T							9	0
„	S								T						9	0
None									1	3	1		2		9	7

S = Start of treatment.
T = End of treatment.

Glycarbylamide is recommended as a drug for prophylactic medication. Under laboratory conditions a test of the anticoccidial properties of a chemical can also be obtained by studying the effects of initiating medication when a later stage of the coccidial life cycle has been reached. The previous experiment has shown that the maximum effect of medication with glycarbylamide is exerted on late schizogony and little difference may be expected between the protection offered by the drug against infections initiated after and those initiated shortly before a term of medication has started. This assumption was tested in the next experiment.

The effect of starting medication after infection.

Experiment 5. Four groups, each of thirteen 7 day-old chickens, were treated as follows: the chickens of all groups were infected with 100,000 sporulated oocysts of *E. tenella.* Three groups were given access to food medicated with 0.003 per cent. w/w glycarbylamide starting 24, 48, and 72 hours after infection, respectively, and lasting until the fourteenth day after the oocysts were administered. The fourth group was infected but received no medication. The results are shown in Table 4.

Concurrently with Experiment 4 an identical experiment was performed using *E. necatrix* as the infective agent.

Experiment 6. Four groups, each of twelve 7 day-old chickens, were infected with 100,000 sporulated oocysts of *E. necatrix.* Three groups received medication as in

Experiment 4, and the fourth group received no medication. The results are summarised in Table 5.

The best results in both experiments were obtained when glycarbylamide medication was not delayed for longer than one day after the oocysts were administered,

TABLE 4

The effect of delaying treatment with glycarbylamide for 1, 2, *and* 3 *days after infecting with oocysts of* E. tenella.

Hours after infection with oocysts that treatment started	Deaths from caecal coccidiosis			Number of chickens	Total deaths from caecal coccidiosis
	Days after infection with oocysts				
	5	6	7		
24				13	0
48	3	1		13	4
72	11	1		13	12
No medication	11	1		13	12

TABLE 5

The effect of delaying treatment with glycarbylamide for 1, 2, *and* 3 *days after infecting with oocysts of* E. necatrix.

Hours after infection with oocysts that treatment started	Deaths from intestinal coccidiosis			No medication Number of chickens	Total deaths from intestinal coccidiosis
	Days after infection with oocysts				
	5	6	7		
24				12	0
48	2	1		12	3
72	3	4		12	7
No medication	4	3	3	12	10

although a delay of two days before starting medication apparently gave much the same protection against both diseases. When medication was delayed for three days little or no protection was afforded against caecal coccidiosis but in this instance protection against the intestinal disease was more marked.

B. E. acervulina.

1. The effect of starting medication before infection.

Experiment 7. Five groups, each of four 9 day-old R.I.R. chickens, were infected with 50,000 oocysts of *E. acervulina.* Groups 1-4 received medicated diets for a period starting 24 hours before and continued for twelve days after inoculation. Group 1 received 0.003 per cent. glycarbylamide, group 2 received 0.005 per cent. glycarbylamide, group 3 received 0.0125 per cent. nicarbazin and group 4 received 0.0125 per cent. sulphaquinoxaline and group 5 received no medication. Total oocyst production of all groups was determined from 4-18 days after infection. The results are given in Table 6.

Experiment 8. Four groups, each of four 15-day-old R.I.R. x W.L. chickens, were infected with 500,000 oocysts of *E. acervulina.* Group 1 received 0.003 per cent. glycarbylamide in the diet, group 2 received 0.0125 per cent. nicarbazin in the diet, group 3 received 0.0125 per cent. sulphaquinoxaline in the diet and group 4 received

no medication. Treatment was started 24 hours before infection in groups 1-3, and was continued until the 12th day after infection. The oocyst-production of each group is given in Table 7.

Sulphaquinoxaline completely suppressed oocyst-production in both experiments whilst nicarbazin gave substantial control. In the first experiment, in which a relatively light infection was used, the protection given by nicarbazin was greater than that given by glycarbylamide and at the lower level. In experiment 2 in which the dose

TABLE 6

Showing the oocyst production of E. acervulina *from* 4-18 *days in groups of* 4 *chickens treated with different coccidiostats in the diet.*

Group Number	Compound tested and concentration in diet		Number of *E. acervulina* oocysts discharged from the 4th-18th day of infection	
			Total per group in millions	Average number per bird in millions
1	Glycarbylamide	0.003%	17.33	4.33
2	"	0.005%	1.77	0.44
3	Nicarbazin	0.0125%	1.55	0.38
4	Sulphaquinoxaline	0.0125%	0	0
5	Untreated		541.39	135.34

TABLE 7

Showing the oocyst production of E. acervulina *from* 4-15 *days after infection in groups of* 4 *chickens treated with glycarbylamide, nicarbazin and sulphaquinoxaline.*

Group Number	Compound tested and concentration in diet		*E. acervulina* oocysts discharged from the 4th-15th day of infection	
			Total per group in millions	Average number per bird in millions
1	Glycarbylamide	0.003%	300.42	75.1
2	Nicarbazin	0.0125%	1.06	0.26
3	Sulphaquinoxaline	0.0125%	0	0
4	Untreated		959.68	239.92

of infective oocysts was raised from 50,000 to 500,000 per bird, nicarbazin gave much better control of the disease than glycarbylamide. However, in another study (in press), we have shown that nicarbazin is not highly active in protecting chickens when the infective dose of *E. acervulina* is increased to proportions which produce mortality in untreated chickens. Considerable variation in individual susceptibility to this infection has been noted and because of this it is difficult to know whether glycarbylamide at 0.003 per cent., which is the effective concentration for the prevention of serious *E. tenella* and *E. necatrix* infections, has a pronounced effect on heavy infestations with *E. acervulina* in all cases.

C. E. meleagrimitis in turkeys.

1. The effect of medication on intestinal coccidiosis of turkeys.

Two experiments were performed to determine what effect, if any, glycarbylamide may have on the course of severe experimentally-induced *E. meleagrimitis* infestations in turkey poults. In the first experiment the activity of the drug was compared with

that of nicarbazin, nitrofurazone and sulphaquinoxaline, and in the second experiment with that of nitrofurazone and nicarbazin only.

Experiment 10. In the first experiment four groups, each of twelve or fourteen 20 day-old poults, were given medicated food. Group 1 received 0.003 per cent. w/w glycarbylamide, group 2 received 0.0125 per cent. w/w nicarbazin, group 3 received 0.0125 per cent. w/w sulphaquinoxaline and group 4 received 0.01 per cent. w/w nitrofurazone. Medication was started two days before each poult was given 200,000 sporulated oocysts of *E. meleagrimitis*. The fifth group (control) received unmedicated food, and was infected at the same time as the others. Treatment was continued until the seventh day after infection with oocysts. All the turkeys died in groups 1, 2, 4 and 5, but no deaths occurred in the group treated with sulphaquinoxaline.

Experiment 11. In the second experiment four groups of twelve, twelve, eleven and nine 17 day-old turkey poults respectively were treated as follows: group 1 received 0.01 per cent. nitrofurazone, group 2 received 0.0125 per cent. nicarbazin, group 3 received 0.008 per cent. glycarbylamide. The drugs were incorporated in the diet in all cases, and medication was continued until the end of the experiment. The medicated diets were fed for two days before the poults were infected with 200,000 sporulated oocysts each. Group 4 received no medication and the poults were given 200,000 sporulated oocysts each. All the poults of every group died from acute intestinal coccidiosis, the majority of deaths occurring on the seventh day of the infection. Under the conditions of the experiments treatment with nicarbazin, nitrofurazone and glycarbylamide failed to control heavy infestations with *E. meleagrimitis*. Sulphaquinoxaline, on the other hand, afforded complete protection against this species.

Discussion

Satisfactory control of caecal (*E. tenella*) and intestinal (*E. necatrix*) coccidiosis was obtained with glycarbylamide. The best results were obtained when medication was started before the infective doses of oocysts were administered and was continued to cover second generation schizogony. Treatment gave excellent results when applied to day-old infections of both species and was continued for fourteen days after the day oocysts were administered. Little or no protection was afforded when treatment was first applied to three day-old infections. The effect of glycarbylamide used at the recommended concentration of 0.003 per cent. in reducing the oocyst-production of *E. acervulina* was inferior to that of nicarbazin and sulphaquinoxaline which in this respect, was by far the most effective compound studied. Glycarbylamide, nicarbazin, and nitrofurazone all failed to control experimentally-induced *E. meleagrimitis* infections in turkeys, whereas sulphaquinoxaline gave complete protection. The inactivity of nicarbazin against *E. meleagrimitis* observed in these experiments differs from the observations of Cuckler *et al* (1956) who recorded that this drug prevented mortality resulting from intestinal coccidiosis caused by this species.

Summary

1. The anticoccidial activity of glycarbylamide was tested against (1) *E. tenella, E. necatrix,* and *E. acervulina* in chickens and (2) against *E. meleagrimitis* in turkeys.

2. Glycarbylamide was effective in protecting chickens from heavy experimentally-induced infections with *E. tenella* and *E. necatrix* but was inferior to sulphaquinoxaline

in reducing the oocyst-production of *E. acervulina* when continuous treatment was started before infection with oocysts.

3. Experimentally-induced intestinal coccidiosis in turkeys caused by *E. meleagrimitis* was not prevented by glycarbylamide, nitrofurazone or nicarbazin. Complete protection was given by sulphaquinoxaline.

Acknowledgments

We wish to thank Messrs. Merck, Sharp and Dohme Ltd., Messrs. May & Baker Ltd., and Messrs. Smith, Kline and French Ltd., for supplying the drugs used in these tests.

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AN UNEXPLAINED EOSINOPHILIA IN THE MEDULLA OF THE ADRENAL OF SHEEP

by

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A varying number of cell types can normally be distinguished in the medulla of the adrenal of the sheep. Of these secretory cells form by far the greatest bulk.

During study of the region for general research purposes a notable feature met with which by virtue of the complete lack of evidence as to its cause might be considered to be pathological, was the appearance in several sections of large aggregations of adult eosinophils.

These eosinophils were arranged around the central vein in clusters of varying density and on occasions these clusters were such as to obscure the parenchymal cells entirely.

Eosinophils were found to be present in the medulla of approximately six per cent. of the 250 adrenals examined. They occurred in the glands of animals of both sexes including castrated males and though an attempt was made by a differential blood count to find a correlation with the physical and sexual condition of the animals concerned, none was uncovered.

As far as the author has been able to determine, this phenomenon has been reported only by Flint (1900) who writes: " In one instance I observed in the medulla of the sheep's adrenal a great number of eosinophilic leucocytes without any evidences of inflammation, and although many sections were cut, the observation was never repeated and the fact remains isolated and unexplained."

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PLATE I

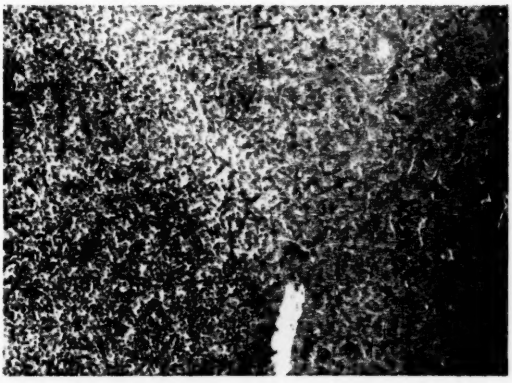

FIG. I

Eosinophils in the medulla of a half-bred wedder lamb. Haematoxylin and eosin. x 130

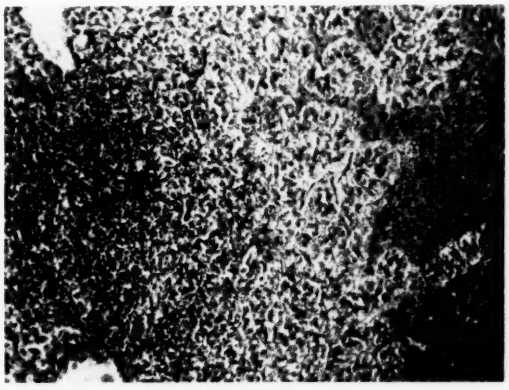

FIG. II

Eosinophils in the medulla of a Blackface ram. Masson's trichrome. x 130

(Photographs by R. S. Hood)

(Article by W. M. Stokoe on page 62)

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PLATE I

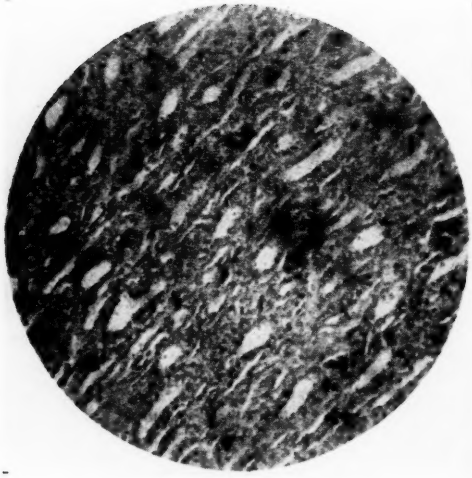

FIG. 1

Kidney from a Vitamin A deficient goat showing degenerated granular material in the lumen of some of the tubules which has taken up haematoxylin stain, suggestive of calcium deposition. Haematoxylin and Eosin x 100.

(Article by B. Dutt, B. N. Majumdar and N. D. Kehar on page 63)

VITAMIN A DEFICIENCY AND URINARY CALCULI IN GOATS

by

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There is a growing awareness of the serious nature of the losses due to urinary calculi in livestock, but the aetiology of the disorder is not fully understood.

Johnson *et al.* (1940) report a 10 per cent. loss due to urinary calculi in one feeding enterprise involving 2600 wethers. Many factors have been suggested by different workers to be responsible for the formation of urinary calculi. Osborne and Mendel (1917) and Higgens (1935) believed that vitamin A deficiency played an important role. Several workers including Beeson *et al.* (1943), Eveleth *et al.* (1939) and Lindley *et al.* (1949) and (1953) have presented evidence that vitamin A deficiency is not involved in calculi formation.

Hard water, mineral imbalance, vitamin A deficiency, infections, hyperparathyroidism, alkaline urine and irritation are the most important factors so far considered.

Eveleth and Millen (1939) suggested mineral imbalance as the most plausible factor and they specify that a high magnesium content in the ration increases the incidence of urinary calculi.

Johnson *et al.* (1940) were unable to produce calculi by feeding a ration, the dry matter of which contained 1.5 per cent. magnesium. They reported that an alkaline urine does not necessarily predispose to calculi formations. Hagger and Magath (1925) stress the importance of bacterial infections in the formation of urinary calculi.

Beeson *et al.* (1943) reported that experimental diets high in linseed and cotton seed meal, Ca, Mg or Ca and P fed to wether lambs with or without an adequate source of vitamin A did not lead to the formation of urinary calculi within 150 to 160 days. Vitamin A deficiency was eliminated as a cause of urinary calculi under the conditions observed in their study. Wheat bran was found to lead to the formation of urinary calculi. Individual variation in the absorption and excretion of dietary minerals was found by the authors to be worthy of consideration in the aetiology of urinary calculi formation.

The report of the Council on Pharmacy and Chemistry to the American Medical Association states that deficiency of vitamin A alone cannot be accepted without reservation as a sufficient condition for the formation of urinary calculi whereas deficiency of vitamin A and D together can be accepted on the evidence advanced to that date. Lindley *et al.* (1949) while investigating the effect of vitamin A deficiency on semen quality and the effect of testosterone and pregnant mare semen on vitamin A deficient rams found, that during the course of the experiment, eight rams died of urinary calculi.

The losses occurred in both vitamin A deficient and supplemented lots. They are of the opinion that some factor of the ration other than its low carotene content is a causative agent. Lindley *et al.* (1953) present data on the effects of higher intake of

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$CaCO_3$, $MgCO_3$, and K_2HPO_4 on the blood and urine contents of calcium, phosphorus, magnesium, urine pH etc. and their relation to urine calculi formation. They reported that under the conditions of their experiment, supplementation with K_2HPO_4 was the cause of highest incidence of calculi. A vitamin A or carotene free diet does not seem to be involved in calculi formation.

Elam *et al.* (1956) studied the effect of minerals and concentrates on the incidence of urinary calculi in sheep. The addition of potassium to the diet of 20 sheep was followed by seven cases of calculi whereas when phosphorus was added there was only one case in 20. When K and P was added to the diet either as potassium acid phosphate or as phosphoric acid and K_2CO_3 calculi developed in 28 out of forty sheep. In forty sheep receiving beet pulp, there were 20 cases of calculi as against 10 cases in 40 sheep receiving no beet pulp.

The effects of carotene free diet on adult goats in relation to urinary calculi are presented in this paper.

Materials and Methods

Adult male goats 2-3 years of age were kept on an almost carotene free diet, but complete in all other respects, for about one year and eight months.

Histopathological studies of tissues obtained from 4 dead goats which had suffered from vitamin A deficient group were carried out. Tissues were fixed in formal saline and embedded in paraffin. They were 5-6 μ and stained by Haematoxylin and Eosin.

Results

Corneal ulceration, unthrifty condition, night blindness, elongated hooves and the loss of hair in patches were the chief characteristic clinical signs exhibited by these goats.

Interesting histopathological changes were observed in the kidneys of all the deficient goats. There was widespread keratinisation of the epithelium of the renal pelvis. The cytoplasm of most of the cells of the tubules was shed and in consequence the cells looked comparatively small. The shed cytoplasm had a granular, reticular or a homogeneous hyaline appearance due to the precipitation of albuminous material during fixation. Some of the tubules contained degenerated granular material. This material had taken up the haematoxylin stain which is suggestive of deposition of calcium salts (fig. 1). Obviously these granules are the remains of the shed and degenerated epithelial cells which have been calcified. The walls of the inter-tubular blood vessels showed hyaline degenerative changes and portions of the walls showed a deposition of calcium salts. It is possible that the changes in the kidneys comprising widespread keratinisation of renal epithelium and the affinity of such degenerative areas for calcium salts are preliminary stages in the formation of urinary calculi but calculi were not found in these animals. Van Leersum (1928) has reported similar histopathological changes in the kidneys of rats kept on vitamin A deficient diet.

Discussion

Hard water, mineral imbalance, vitamin A deficiency, infection, hyperparathyroidism, alkaline urine and irritation are factors which have been suggested by different workers to be responsible for or associated with urinary calculi. From the results of this experiment it appears that vitamin A deficiency may predispose to the formation of urinary calculi. This concept is supported by histological examination of the

kidneys which showed that calcium salts were deposited in the renal tubules and around the blood vessels. It is probable that if the deficiency state had been prolonged, or if it had been complicated, for instance by vitamin D deficiency, definite stone formation would have taken place. Histological examination of the kidneys gives the earliest indication of the possibility of stone formation in Vitamin A deficient goats.

Summary

Adult male goats of 2-3 years of age were kept on almost carotene free diet but complete in all other respects for about 1 year and eight months. Corneal ulceration, unthrifty condition, night blindness, elongated hooves and loss of hair in patches were the chief clinical signs exhibited by these goats.

Histopathological examination of kidneys of vitamin A deficient goats revealed widespread keratinisation of renal epithelium. Some of the renal tubules of the pelvis contained shed and degenerated epithelial cells. These degenerated cells had taken up the haematoxylin stain which is suggestive of calcium deposition. From the observations made in the present experiment, it is suggested that vitamin A deficiency predisposes to urinary calculi formation. There may be other factors which precipitate the development of calculi in Vitamin A deficient goats.

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REVIEW

REPORT ON THE ANIMAL HEALTH SERVICE IN GREAT BRITAIN 1956. Including Report of Proceedings under the Diseases of Animals Act, 1950. *H.M.S.O.* 1958. 6/-.

FIVE parts are included in this report which covers accounts of animal diseases, the regulations designed to prevent the introduction and spread of animal disease, the protection of animals in transit and the export of livestock, animal products and other materials. Part 5 is concerned with the laboratory, research and investigation services.

There was an increase in the incidence of Foot and Mouth disease in Great Britain in 1956. Whereas there were 9 outbreaks in 1955, 162 occurred during the year of 1956. Thirty two of the outbreaks were primary ones and of these 13 were probably from imported meat or bones. Of the 32 outbreaks, 10 were due to the "O" type of virus; 14 were type "C" and 7 were type "A"; one was not identified.

The number of confirmed outbreaks of swine fever was lower than at any time since 1950; there was a continued slight occurrence of atrophic rhinitis among Landrace pigs.

Well over half of the outbreaks of fowl pest were in Lancashire and the incidence throughout the country was a little higher in 1956 than in 1955. The compensation paid in 1956 however for this alone amounted to £1,371,710.

Anthrax was disturbingly prevalent and there were 1,245 outbreaks, which is a higher number than any recorded since 1910. It was widespread in its occurrence

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and, as expected, the sources of infection were mainly feedingstuffs and fertilisers, and the incidence of the disease was highest in February and March.

As expected, warble fly infestation increased because of the fine summer during the previous year. An experiment in the Isle of Wight demonstrated the great reduction of warble fly infestation which can be achieved. The methods employed were the regular and thorough inspection, and dressing of cattle under supervision, in a self contained and manageable area. This is to be continued during 1957.

The practice of the slaughter of cattle infected with *Brucella melitensis,* which has continued since 1940, is now abandoned. It was decided towards the end of 1956 to discontinue it after discussions with the Ministry of Health and the Department of Health for Scotland.

The regulations which are devised to prevent the introdutcion and spread of disease in this country are commented upon briefly. Those concerned with the export of livestock, animal products and other materials form another section of this report whilst the methods employed for the protection of animals in transit are not neglected.

The laboratory, research and investigation services cover a wide field and accounts are given of the many and varied studies into subjects ranging from Brucellosis, Vibriosis, Tuberculosis and Johne's disease to some of the less well known conditions which are now being reported especially in poultry. There are accounts of the work of the artificial insemination centres at Reading and Ruthin and there are statistical tables of great variety. There is also a long list of the publications of members of staff and this alone gives some indication of the scope of this worthy and satisfying report.

With regard to tuberculosis the situation is in a very healthy state for by the end of 1956, nearly 7 million cattle were attested. This constitutes about 67 per cent. of the cattle in Great Britain and shows that the eradication of bovine tuberculosis from this country is making steady and sustained progress.

NOTICES

'MIXTAMYCIN' SOLUTION VETERINARY

The Distillers Company (Biochemicals) Limited announces the introduction of the above new veterinary product. Each vial contains 5.0 grammes streptomycin sulphate and 5.0 grammes dihydrostreptomycin sulphate in 30 ml. solution ready for injection.

Pack and prices are as follows:—

		Price to Veterinary Surgeons
'MIXTAMYCIN' SOLUTION VETERINARY 30 ml. vial	With order of less than £30 value ...	12s. 4⅞d.
	„ „ „ £30 value and over ...	11s. 7½d.
	„ „ „ £100 „ „ „ ...	11s. 4⅛d.

CHLOROMYCETIN VETRETTES, 500 mg.

With effect from January 19th, 1959, this new Parke, Davis veterinary product for oral and intrauterine administration to large and small animals will be available from Parke, Davis & Company, Hounslow, Middlesex.

Chloromycetin Vetrettes, 500 mg. are supplied in boxes of six, at a list price of 18s. 0d. per box, subject to usual discount.

GLAXO LABORATORIES LTD. announce that a 30 ml. vial of Veterinary Cytamen '250' will be available on the home market as from 2nd February, 1959.